THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

66TH STREET AND YORK AVENUE
NEW YORK 21, N.Y.

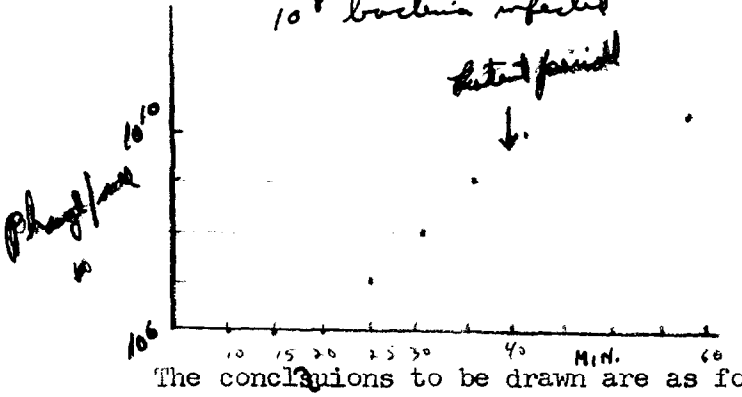
July 13,1953

Dear Joshua:

I received the cultures and the set of lysogenicity reprints which I have distributed as per names. It has been unbearably hot but with the mice away for the summer and my time to myself have been getting some work done.

As for tricks to inactivate phage-FA , the ultra-sonics works fine with sufficient treatment; undetectible to 4 minutes, 20 % at 4 minutes and 60% at 8 minutes. What is really needed is some method which will inactivate one or the other , a treatment in which the adsorption mechanism is not the limiting factor.

Incorporation or association of phage and FA. I have repeated the premature lysate results using ultra-sonics to disrupt the bacteria and the results agree with those previously obtained. Let me give you the details: 22V was grown on A- B+ cells and then infected A+B- cells at a multiplicity of three. Samples were incubated and then rapidly chilled at the appropriate time and then treated with US. Samples were assayed and also concentrated by vacuum distillation for more precision in the transduction assays.



Min	Phage to FA ratio A+	B+
60	$2\times10^6/1$	$\geq 10^{10}/1$
40	$4\times10^7/1$	
* 40 supernatant	$+25\times10^6/1$	
35	$\geq 4\times10^9/1$	
0	$\geq 10^{10}/1$	$10^7/1$

The conclusions to be drawn are as follows: 1) FA becomes associated with only the late maturing phage particles or only with those particles released by natural lysis. The latter hypothesis would make it a surface adsorption while the former makes more sense. The data on hand do not differentiate the two because of the lack of precision in transduction assays when the phage to FA ratio goes above 10^8 to one, as only a finite number of particles can be adsorbed per bacterium and to have sufficient bacteria there is usually too high a background of spontaneous reversion. What is needed is a good technique of lysis inhibition to determine whether once FA appears amongst the phage the ratio increases exponentially as the phage . 2) if phage adsorption is complete there is no detectible carry over of genetic activity from previous host , certainly less than could be explained by the factor of dilution, this is again, and even more so, complicated by the reversions etc.

I hope to do some host phosphorus labeling experiments shortly to see whether the chemical data will follow the biological. Since one can have as high as a factor of one hundred difference in the activity of two crops of phage there is some hope of detectible differences. However as the difference between early and late phage may be a matter of size rather than amount of host nucleic acid incorporated the labeling of phage progeny could be almost anything.

* cells sedimented away and supernatant assayed before ab.l.

Lysogenization-protection: One of the problems that has long been bothering me is why the transduction assays on phage sensitive bacteria (22) are linear. If one does a simple infection experiment at multiplicity of one only 5-10 % percent of bacteria become lysogenic the rest lyse or are uninfected. Those that become lysogenic could almost be accounted for on the basis of multiple infection. Therefore on theoretical grounds I've never understood how your lysogenisis-protection experiment worked and when I tried it, it did not, as I expected it wouldn't, although this still left me in a quandary as to why the transductions survived in the first place. We did the experiment differently and this at least provides some clues as to what happens. While you plated after infection with the phage selecting phage, I superinfected incubated for ten minutes further and then plated. The experiment was accomplished by both methods and we both are essentially right.

	Transductions	Clones (Recovery %)
Plate infection	45%	100% (not mottled)
Tube infection	7% mottled	10% includes mottled + roughs

There are several points that need explanation and I venture these guesses. The fact that the super-infected tranductions survive when plated with phage is an artifact due late or negligible superinfection under these conditions. The fact that all 22 infected clones survive is due to the tremendous superinfection perhaps setting up conditions analogous to multiple 22 (alone) infection. The fact that neither transductions nor clones survive when superinfected in adsorption tube means that as per usual only a small fraction of singly infected cells become lysogenic. This all leaves the dilemma of why the transductions survive in the first place. It seems possible that cells infected on a non-growth medium and left on such a medium may have a higher probability of becoming lysogenized in time if not previously superinfected with more virulent phage. There is something here, but I'm not quite sure I see it yet. Anyway it is interesting how science can twist itself to suit the experimenters preconceptions, you were sure the experiment would work and chose those means that make it so , I on the other hand didn't believe it and chose again the proper method.

Purification of phage. Have cleaned up a batch of 22V by several cycles of differential centrifugation and have a nice clean preparation assaying close to 10^{13} per ml . Gives lovely U.V. adsorption curves. May have less nucleic acid per phage than the Ts but this will have to await more precise determination of the size of the particle . Have sent some to Williams for some micro-graphs and also will take some here.

Double transductions. With the possibility of getting transduction of 950 H- about 1/1000(100 particles per bacterium) a search for doubles became feasible and am cooking up a big batch of 22 for this purpose as would provide more direct evidence that as many as one hundred particles can participate in the transduction of a single bacterium, also some estimates of bacterial competence etc.

I shall be going out to CSH in about two weeks to help with the course in microbial genetics especially the coli recombination. They have a recording of Hayes' talk which they are going to use. I don't feel in a position to agree with or refute his arguments and would appreciate a brief statement on your current views on the matter. I ask this not because I want to commit you but rather that anything I say about coli etc is taken directly from you (I have not become dissassociated from you as yet) and therefore I generally keep my mouth shut about coli. However I am now in a position where I must say something and since it will be confused for yourself I might as well have some inkling of your thinking. Personally I was very much impressed with the streptomycin effect on F+ by F+ crosses and his Hfr mutant. Do you agree that mating is solely between F+ and F- and what is your feeling on the timing of the elimination (vector aside).

These seem to me to be the critical questions.

Dr. A. Braun works just down the hall from me and recently showed me some preparations from his best star-forming strains. Really beautiful. Have decided to take a fling at putting some markers on them. Seems to be just crying for genetic analysis. Unfortunately the strains are extremely resistant to penicillin (2000 units per ml), but shall try any way.

My best to everybody at U.W.

Sincerely,

Austin